basis for helping cells cope and survive ever-changing environments. In this work, we profile the heterogeneity of protein expression across the entire Escherichia coli proteome. Our novel approach integrates live-cell single-molecule microscopy with a high-throughput microfluidic platform to systematically reveal noise properties, localization, and functions in the E. coli proteome.

We have constructed chromosomal fluorescent protein fusions for over 1,000 ORFs from the E. coli genome by an efficient, low-cost conversion of an existing Sequential Peptide Affinity tag library. We have developed a microfluidic platform for high-throughput fluorescence microscopy, coupled with automated imaging analysis, enabling us to record the protein expression of over 100,000 cells per hour, which is sufficient to describe the statistics of about 100 different reporter strains. We have measured the distribution of protein expression across cell populations and determined the noise properties of each gene with single molecule sensitivity as necessary. In addition, we have imaged the localization of proteins to the membrane, cytoplasm, and DNA.

To determine possible factors affecting the noise of specific genes, we correlate our protein expression data with biological markers and other global data sets. We find that a substantial fraction of the proteome is expressed at low copy numbers, in agreement with previous predictions, and these genes are subject to high values of noise. We also observe global properties of protein noise in E. coli and find differences in the scaling between noise and average expression for proteins present at low or high copy numbers. Our data provides the first comprehensive proteomic resource of expression levels and noise with high sensitivity for the model organism E. coli.

EPR Spectroscopy

1575-Pos Board B419

Multifrequency Pulsed EPR investigation of Fe-histidine Interaction of the Uniquely Coordinated [2Fe-2S] Cluster in the Outer Mitochondrial Membrane Protein, MitoNEET

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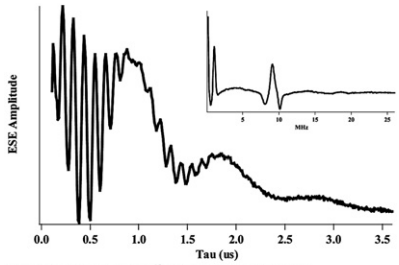
Complimentary pulsed EPR techniques (ESEEM, ENDOR, HYSCORE), over multiple frequencies (X, Ka, Q-bands), were used to characterize bonding interactions of the [2Fe-2S] redox active center of the Outer Mitochondrial Membrane protein, MitoNEET. MitoNEET is the first example of a 3Cys-1His coordinated [2Fe-2S] cluster containing protein. Specifically targeting the uniquely single Fe-histidine interaction, EPR investigations integrated both natural abundance ^{14}N and isotopically labeled ^{15}N protein to determine the hyperfine tensor of a strongly coupled imidazole nitrogen of the bound histidine ligand. 1D-ESEEM experiments in the 31, 35GHz frequency region resulted in deep modulation patterns indicative of being near the ''exact cancellation'' limit and was favorable for a more direct spectral assignment of nuclear quadrupolar transition frequencies. Assignment of His87 as the bound ligand was supported by parallel experiments using H87C mutant. An additional advantage of these higher field experiments allows for greater resolved g-anisotropy and a finer degree of orientation-selected experiments, in progress. These should provide a more accurate description of the [2Fe-2S] ligand bonding interaction important for understanding the electronic structure of this new class of redox active proteins.



2-Pulse ESEEM time domain ^{15}N labeled MitoNEET protein (Inset is Fourier Transform-frequency domain) Data taken at CalEPR Center, UC Davis

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Comparing the Structural Topology and Dynamic Properties of a Model Peripheral Membrane Peptide Magainin-2 Utilizing X- and Q-Band EPR Spectroscopy

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Probing the structural and dynamic properties of membrane proteins poses a very difficult problem due to their hydrophobic amino-acid composition and lipid environment they are associated with. To unravel this dilemma lipid membrane mimics have been used to establish a medium by which membrane proteins can be studied. Magnetically aligned phospholipid bilayers (bicelles) coupled with magnetic resonance spectroscopy can be used to extract pertinent information related to their structural topology. This information can be obtained by aligning the samples with respect to the static magnetic field and measuring the corresponding anisotropic spectral parameters. Our lab uses both solid-state NMR and spin-label EPR spectroscopy to study membrane proteins. EPR spectroscopy offers unique advantages over NMR spectroscopy due to a higher sensitivity and a different frequency domain for probing dynamics. These facts have led us to perform EPR spectroscopic alignment studies on the surface peptide magainin-2, which has been shown to exhibit antimicrobial activity by pore formation in two different frequency domains X-Band (9 GHz) and Q-Band (34 GHz). New and unique EPR lineshapes were obtained which not only elegantly contrast integral and peripheral peptide topologies, but also have implications for further elucidating antimicrobial dynamics and their corresponding mechanisms.

1577-Pos Board B421

Crystalline Spin-Labeled Hemoglobin as a Model to Compare Distances Measured by DEER Spectroscopy and X-Ray Crystallography

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We have recently crystallized and resolved the structure of spin-labeled hemoglobin, while simultaneously using double electron-electron resonance (DEER) spectroscopy to measure inter-spin-label distances within these crystals. Previously, no spin-labeled protein has been analyzed by both DEER-spectroscopy and X-ray crystallography to determine whether the two techniques are in good agreement. Human hemoglobin (Hb) is a useful model system for this comparison, as it readily crystallizes and reacts specifically with the maleimide-TEMPO spin-label (MSL) at Cys93, found within the β subunits of the $\alpha_2\beta_2$ hemoglobin tetramer. For our experiments, we have generated two crystal populations. The first consisted entirely of paramagnetic, EPR-active MSL-Hb, which was used in our X-ray crystallography experiments. The second population contained a low concentration of MSL-Hb in a large excess of hemoglobin labeled with an EPR-silent MSL analog, which assured that distances measured by DEER spectroscopy would not be altered by dipolar interactions between spin-labels of adjacent Hb tetramers. Our results show that both techniques yield similar inter-spin distance measurements, provided that certain precautions are taken to avoid EPR artifacts. We have varied DEER acquisition parameters, such as the dipolar evolution time and acquisition temperature, and analysis methods that affect the accuracy and precision of distance distributions observed by DEER spectroscopy, as compared to those obtained by X-ray crystallography. These results provide the most rigorous analysis to date of the reliability of EPR-based distance measurements. This work was supported by NIH grants (GM27906, AR32961, AG26160, RR22362, GM08700).

1578-Pos Board B422

Structure of the cdb3-ankD34 Complex from Site Directed Spin Labeling Studies

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The spectin-based membrane skeleton is responsible for the remarkable mechanical stability and the unique viscoelastisity of the erythrocyte membrane, which are both essential for the survival of red blood cells in the circulatory system. One of the major junctional sites that links the membrane skeleton to the plasma membrane is a protein complex formed by the cytoplasmic domain of band3 (cdb3) and ankyrinR. In this study, site directed spin labeling (SDSL) has been utilized to investigate the global structure of the complex formed between cdb3 and ankD34 (ankyrin repeats 13-24 of full length ankyrinR). We first characterized physicochemical properties of the complex using gel permeation chromatography and sucrose-gradient sedimentation and determined the stoichiometry of the complex to be one cdb3 dimer bound to two ankD34s *in vitro*. For a series of surface sites residing on the binding interface of cdb3, spin label